MATERIALS AND METHODS FOR ACHIEVING DIFFERENTIAL LYSIS OF MIXTURES WITH THE AID OF ALKALINE LYSIS AND PRESSURE CYCLING TECHNOLOGY (PCT)

CROSS-REFERENCE TO A RELATED APPLICATION

This application claims the benefit of U.S. provisional application Ser. No. 61/941,201, filed Feb. 18, 2014, which is incorporated herein by reference in its entirety.

This invention was made with government support under contract number 2011-ne-bx-k550 awarded by the National Institutes of Justice. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Since its discovery in 1984 by Sir Alec Jeffreys [9], DNA fingerprinting has become an indispensable tool in applications ranging from paternity testing [1], criminal investigations [12], to the study of genetic disorders [9]. The application of DNA technology in the criminal justice system has resulted in an unprecedented expansion in the capabilities of forensic laboratories for the detection of violent crimes such as rape and murder. With improvements in automation, the procedure has expanded to permit determination of property crimes and misdemeanors as well. However, as the number of applications for DNA technology has expanded, inevitable backlogs have occurred due to increasing sample loads. Factors such as time lapse between the incident and sample recovery [10], exposure to external elements [3, 5], and storage conditions, may all result in sample degradation. Hence it is critical to be able to efficiently recover and reliably analyze the evidence collected.

Traditionally, cotton swabs have been used for collection of biological samples such as body fluids, touch samples, and other trace evidence. Despite being a common tool for sample collection, sample extraction from these swabs can be challenging due to strong adherence of the sample to the matrix. Often the bulk of a collected biological sample remains entrapped in the cotton fibers even after elution. This results in a loss of precious evidence [14]. For sexual assault casework in which there is a mixture of body fluids present, there are two challenges. First, the evidence is often overwhelmed with the victim's vaginal epithelial cells and secondly the poor recovery from the swab can often make it difficult to recover the suspect male DNA profile [14, 19].

There have been a number of studies to develop methods to increase the efficiency of cell recovery from cotton swabs. Enzymatic methods are based on the hypothesis that upon digestion of cotton fibers with cellulase enzyme will improve the release of cells from the swab [19]. Alternatively, appropriate detergents or buffers may improve recovery of DNA from the cotton swabs [14]. Different types of swabs and substrates have also been proposed to improve elution of DNA [2].

Another issue is the selectivity of the extraction of DNA. When performing differential extraction of sperm and epithelial cells, it is critical to obtain a clean, unambiguous male profile. This is done by lysing the female epithelial cells with detergent followed by wash steps to isolate the sperm cells. This step is followed by treatment of the sperm cells with detergent and a reducing agent such as dithiothreitol to lyse disulfide bonds in sperm protamine and release the DNA. [6]. Because the procedure is difficult and time consuming, involving multiple wash steps, its recovery is sometimes very poor and user dependent [20]. For example, in a collaborative study by Vuichard, it was found that losses up to 98% of the male DNA were seen in a standard differential extraction and in 30% of these extractions more male DNA was found in the epithelial fraction than the sperm fraction [20].

Because of these problems, a number of alternative procedures have been attempted to permit differential detection of male and female DNA. These include laser microdissection[4], removal of female DNA through alkaline lysis and DNase digestion[8], microfluidics [7] and flow cytometry [16, 17].

Although there have been a number of efforts to improve either the recovery or the extraction of DNA from swabs taken following a sexual assault, the ability to achieve both would be desirable, especially for situations in which sample is limited. Though the organic differential extraction method developed by Gill et al. is still a gold standard in analyzing mixtures [21, 23], its shortcomings create the need for an efficient, quick, and reliable method that can successfully separate DNA profiles in a mixture without compromising sample recovery.

BRIEF SUMMARY

The subject invention provides a two-step protocol using pressure cycling technology (PCT) and alkaline lysis for differential extraction of mixtures. In a preferred embodiment the procedure is used in forensic DNA applications such as, for example, DNA testing in the case of rape.

In accordance with the subject invention, it was found that, for mixtures of sperm and female epithelial cells, pressure treatment in the presence of alkaline conditions resulted in a near complete recovery of female epithelial DNA. Following this pressure treatment, exposing the composition to alkaline conditions at higher temperatures results in selective recovery of sperm DNA with minimal contamination from female epithelial cells.

In one embodiment, the selective recovery of sperm DNA was optimized by examining the effect of sodium hydroxide concentration, incubation temperature and time. 69±6% of sperm DNA was recovered from prepared mixtures in the presence of 0.4 N NaOH at 95° C. for 5 minutes. Following alkaline lysis, the samples were neutralized with 2 M Tris (pH 7.5) and purified with phenol-chloroform-isoamyl alcohol to permit downstream analysis. The total processing time to remove both fractions from the swab was less than 30 minutes. Short tandem repeat (STR) analysis of these fractions obtained from PCT treatment and alkaline lysis generated clean profiles of female epithelial DNA and male sperm DNA, respectively.

By reducing the time for the recovery of DNA from sexual assault mixtures, this new method improves the efficiency of current differential extraction techniques, aiding in effective processing of forensic casework.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** illustrates the effect of pressure treatment on DNA recovery from mixtures in the presence of 0.4 N NaOH solution.

FIG. **2** is a flowchart depicting the protocol for differential extraction of mixtures using alkaline lysis and pressure cycling technology.

FIGS. **3**A-**3**C illustrates Powerplex® 16 HS products of mixture, sperm control, epithelial fraction (post-PCT puri-